THE COUNCIL FOR TOBACCO RESEARCH – U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☐ Second Renewal ☒ Date: June 20,1975

1. Principal Investigator (give title and degrees):

Branislav Vidic, S.D., Professor of Anatomy, Department of Anatomy, Georgetown University School of Medicine and Dentistry

2. Institution & address:

Georgetown University School of Medicine and Dentistry, 3900 Resevoir Rd., N.W., Washington, D.C. 20007

3. Department(s) where research will be done or collaboration provided:

Department of Anatomy and Department of Physiology & Biophysics

4. Short title of study:

The Effect of Cigarette Smoke on Lung Metabolism

5. Proposed renewal date: January 1, 1976

6. How results to date have changed earlier specific research aims:

Because of technical delay in the initiation of smoking experiments, the project outlined in design II is slightly behind schedule. On the other hand, we have begun preparatory work of design IIImuch ahead of the planned starting date.

7. How results to date have changed earlier working hypothesis:

The working hypothesis remains unchanged.

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8. Any additional facilities now required? Describe briefly:

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

See Appended Page 1

10. Append outline of experimental protocol for ensuing year. See description of designs II and III

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

See Appended Page 2

12. Summary progress report (append in standard form as separate document, unless recently submitted).

10035458 33

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Branislav Vidić, S.D.	30	
Paul Hamosh, M.D.	10	
Margit Hamosh, Ph.D.	100	23,900*
Research Associate for electron microscopy (to be named)	100	14,136*

* Include 16.7% (1/1/76 - 6/30/76) & 20% (7/1/76 - 12/31/76) Fringe Benefits

Technical

Sub-Total for A	38,036

B. Consumable supplies (by major categories)

Experimental animals	3,850
Chemicals for ultrastructural studies and photographic supplies	2,200
Radioactive isotopes	1,100
Chemicals for cellular culture and biochemical studies	1,650
Sub-Total for B	8,800

C. Other expenses (itemize)

Maintenance of equipment	1,500
Travel	1,000
Sub-Total for C	2,500
Running Total of A + B + C	49,336

D. Permanent equipment (itemize)

None

Sub-Total for D	

O.K PRB

E. Indirect costs (15% of A+B+C) RSKY

E	7,400
Total request	56,736

10035458 34

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14. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Cellular and Immunological Basis of Pulmonary Diseases (co-investigator)	NHLI 3294-208	$250.000	5/1/74- 4/30/79
The effect of pulmonary blood flow on surfactant synthesis	Washington Heart Assoc 3282-752	$10.350	1/1/75-31/12/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Branislav Vidic, S.D.

Signature [signature] Date 6/20/75

Telephone 202 (Area Code) 625-7527 (Number) (Extension)

Checks payable to

Georgetown University

Mailing address for check: Sam A. Kimble
Georgetown University, 37th &
O Streets, N.W., Washington,
D.C. 20007

Responsible officer of institution

Typed Name Sam A. Kimble

Title Administr. of Sponsored Programs

Signature [signature] Date 7/3/75

Telephone 202 (Area Code) 625 - 3151 (Number) (Extension)

10035458 35

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The year following, emphasis will be on projects outlined in designs II and III. While the biochemical studies remain in the hands of M. Hamosh, Ph.D., the most of the forthcoming technical and semi-technical involvement concerns the structural studies including: conventional transmission microscopy of isolated cells and intact lung pieces, scanning studies of isolated cell, and the auto-radiographic studies of cells in situ and isolated cells following administration of 3H palmitic acid. Furthermore, the principal investigator is planning to undertake major involvement in structural studies related to the Program Project, therefore we made the following redistribution in the salary category: Part of the biochemical investigations presently conducted by Y. Shechter, Ph.D., will be continued under the supervision of M. Hamosh, Ph.D., who is in charge of our biochemical laboratories. The funds made available will be conveyed toward hiring a competent electron microscopist, knowledgable in cytochemical techniques required for our studies: Dr. J.T. Wolosewick, University of Colorado at Boulder, Dr. Keith Porter Laboratory, Department of Mollecular, Cellular & Development Biology, Boulder, Colorado 80302.

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Vidić, B., and Tandler, B. Ultrastructure of the Secretory Cells of the Submucosal Glands in the Human Maxillary Sinus. submitted to J. of Morph. (see attached manuscript).

Vidić, B., Interaction of Membranous Phospholipid Layers During Coalescence Between Some Secretory Granules (Abstract accepted for Proceedings of the Tenth International Congress of Anatomists, Tokyo, Japan, August, 1975). (see attached manuscript).

Vidić, B., Hamosh, M., and Hamosh, P. Mucopolysacchrides in the Pulmonary Surfactant Hypophase. An Electron Microscopical Study of Rat Lungs Treated with Ruthemium Red. submitted to J. Cell Biol. (see attached manuscript).

Vidić, B., Hamosh, M., Blunda, M., and Hamosh, P. Synthesis of Surfactant as a Function of Ventilation (in preparation).

Vidić, B., Compton, S., Wrathall, J. and Hamosh, M. Synthesis of Surfactant by Isolated Type II Pneumocyte (in preparation).

Vidić, B., and Hand, A. (NIH, NIDR) Interaction of Membranes during the Coalescence among the Secretory Granules of Human Upper Respiratory Glands (in preparation).

Vidić, B., Secretory pathways in Rat Tracheal Goblet Cell. Mechanism of Secretory Granule Release (to be submitted to J. Cell Biol.).

Hamosh, M. and Hamosh, P. The Effect of Estrogen on the Lipoprotein Lipase Activity of Rat Adipose Tissue. J. Clin. Invest. 55: 1132, 1975

Hamosh, M. and Hand, A.R. Development of Secretory Activity in Rat Lingual Serous Glands. J. Dent. Res. 54: 168,1975

Hamosh, M. and Hamosh, P. Lipoprotein Lipase in Rat Lung, the Effect of Fasting. Biochim. Biophys. Acta. 380: 132, 1975

Hamosh, M., Yeager, H. Jr., Schechter, Y., Blunda, M., Vidic, B. and Hamosh, P. Lipoprotein Lipase in Rat Lung, The Effect of Hormones (in preparation)

10035458 37

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SUMMARY PROGRESS REPORT

Vidic, B., and Tandler, B. Ultrastructure of the Secretory Cell of the Submucosal Gland in the Human Trachea. [illegible]

In our previous report we indicated changes concerning slightly delayed progress of designs I and II and early initiation of design III. In Our group has been joined by J. Wrathall, Ph.D. and Miss S. Compton, B.S. from our Department. Their expertise in the tissue culture techniques enabled us to quickly advance our knowledge about the biological changes related to isolation, short-term, and long-term cultured type II pneumocyte. Results until now indicate that the isolation of type II cell from young postnatal rats following mincing of the lung, trypsin application and subsequent centrifugation of cell suspension over 2M sucrose is successful. We find that in early stages of culturing, type II cells deplete most of the lamellar bodies. This phenomenon is perhaps caused by osmotic changes, or by some additional conditions, in transferring the cell from in vivo to an in vitro situation. Furthermore, some of the most recent findings of early cultured type II cell demonstrate varying quantities of intracellular fat droplets, which are never observed in an in vivo condition. Our hypothesis supports the view that the fat droplets (see figures 1-4) at this stage of cell culture might represent the end-product of phospolipolytic enzyme activity upon the preexisting phospholipids contained with the lamellar bodies and/or mitochondria.

In order to test this hypothesis we plan to assay these cells for acid phosphatase activity, and possibly for phospholipase content according to biochemical procedures. Another aspect related to the cultured situation is the cellular response to corticosteroid treatment, a condition that is extensively studied in human, perinatal pulmonary pathology. Our data derived from the treatment of cells with different doses (0.1 mg/ml - 2.0 mg.ml) of dexamethasone suggest that the drug slightly increases the lecithin content inside the cell (see Tables I and II). These preliminary observations will be expanded in a systematic way to study the ratio of dipalmitoyllecithin (DPL) synthesis by type II cell as a function of hormonal influence in a "clean" and a nicotine-containing environment. It is also hoped that a better methodology will be established to test the surface tension activity of the extracellular medium.

[illegible] Type II [illegible] the Effect of Hormones

Our previous autoradiographic studies with 3H palmitic acid incorporation into DPL indicated a dependence of surfactant synthesis and/or excretion from the cell on the tidal volume of ventilation. Because in the first experiments, the time intervals at which the tissue samples were fixed for autoradiography were too distant, another extensive experiment was undertaken sometime early this year. In the latter experiment, the tissue samples were collected at 1, 3, and 5 minute intervals following the injection of the isotope.

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SUMMARY PROGRESS REPORT

Like the previous experiments, the amount of palmitic acid in type II cells was greater in hyperventilated than in normal-ventilated and still greater than in non-ventilated lungs. It was ascertained that the earliest appearance of labelled fatty acid on the alveolar cell surface of type II cell and/or on the surfactant layer of the alveolus occurs within 3 minutes after the injection of the label. In view of these findings we are projecting some other techniques, in addition to ones already in use, for projects I and II. Because information is needed on the nature of label in the alveolar space and in the continuous surfactant l ining layer at different time intervals, all of the future experiments will include the count of radioactive content from the lavaged lungs also. This additional information will provide a meaningful support to the analytical studies on the rate of surfactant synthesis as a function of ventilatory volume in control and experimentally "smoked" animals.

In preparation for our studies on the effect of tobacco smoke on lung metabolism we have first investigated lung metabolism in normal rats. Since there is in general a lack of standardization of the methodology employed in the study of lung metabolism we have adapted the following methods to the study of lung metabolism in tissue slices:

1. Glucose Metabolism measured as the amount of U ^{14}C Glucose metabolized to $^{14}CO_2$ (aerobic metabolism), lactate (glycolysis) and incorporated into tissue lipid.
2. Protein Synthesis measured by the incorporation of labeled leucine into lung protein.
3. Glucoprotein Synthesis measured by the incorporation of glucosamine.
4. Lipid and Phospholipid Synthesis measured by the uptake and esterification of labeled palmitate.

We have chosen the tissue slice method since it is the least time consuming and according to recent studies has an identical glucose metabolism as that of the perfused lung.

We have adapted the following analytical methods to the study of lung composition:

RNA, DNA, protein, lipid analysis (separation, identification and quantitation of the individual components of the major lipid classes-phospholipids and neutral glycerides).

We have at present completed 2 series of experiments in which rats were

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exposed to the smoke of Kentucky reference cigarettes IAI or IRI. Exposure was twice daily, 7 days a week for 4 weeks. Each run contained 4 groups of 6-8 rats each:
Group I 1 cigarette twice daily
Group II,3 cigarettes twice daily
Group III control machine exposed
Group IV control- same batch of rats as Groups I-III, but never in contact with the smoking machine.
We are now processing our data for experiments with IAI cigarettes (the experiments with IRI cigarettes are still in progress). Preliminary experiments with IAI cigarettes indicate:
1. Palmitate incorporation and esterification was not affected by exposure to cigarette smoke.
2. There is a slight decrease in leucine incorporation into protein in the 3 cigarette exposed rats.
3. There is an increase in glucose oxydation after 1 or 3 cigarette exposure.
4. There is a marked increase in glucoprotein synthesis in rats exposed to 1 or 3 cigarettes.
There are slight differences between the two control groups, indicating that exposure of the animals to the smoking machine (without smoking) affects their metabolism.

We have initiated experiments on the biochemistry of the developing lung. These experiments are carried out in rabbits (late fetal, neonate and throughout the developmental period). The rabbits are a gift from Dr. Bellanti (Dep. of Pediatrics) who is studying the development of defense mechanisms in the lung. This study will therefore investigate if there is any correlation between "biochemical maturation" and development of immune competence in the lung.

We have continued our studies on lipoprotein lipase activity in the lung, with special emphasis on the effect of hormones known to affect the enzyme activity in other tissues. Our results indicate that of a number of hormones tested only dexamethasone caused a change in enzyme activity-stimulation. The increased demand for palmitic acid needed for the steriod stimulated surfactant synthesis could therefore be met by an increased uptake of triglyceride-fatty acids, which is regulated by

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lipoprotein lipase. The manuscript is now in preparation As part of this study on lipoprotein lipase activity we made the interesting observation that estrogen administration markedly decreases lipoprotein lipase activity in adipose tissue of male and of ovariectomized rats. (See appended reprint).

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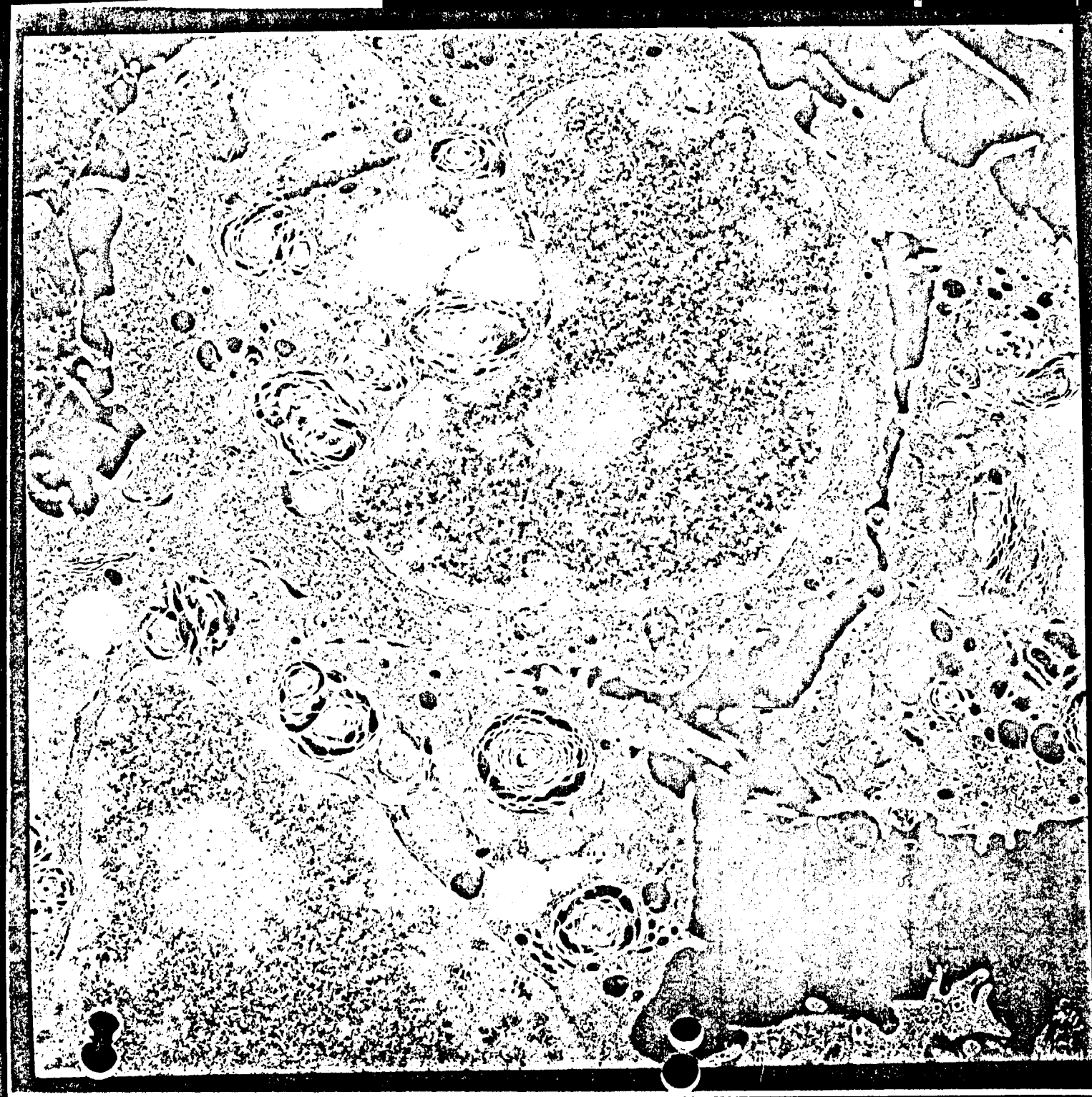

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CELLS

	CONTROL	0.1 MICROGRAMS DEXAMETHASONE	2.0 MICROGRAMS DEXAMETHASONE
ORIGIN + LYSOLECITHIN	2% 494 CPM	16% 13383 CPM	7% 1582 CPM
LECITHIN	17% 5231 CPM	38% 31783 CPM	9% 2056 CPM
PE	3% 814 CPM	4% 3680 CPM	3% 586 CPM
NEUTRAL FATS	78% 22666 CPM	42% 35045 CPM	81% 18348 CPM
	29205 CPM TOTAL	83891 CPM TOTAL	22572 CPM TOTAL

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MEDIA

	CONTROL	0.1 MICROGRAMS DEXAMETHASONE	2.0 MICROGRAMS DEXAMETHASONE
ORIGIN + LYSOLECITHIN	0% 0 CPM	1% 1671 CPM	1% 57 CPM
LECITHIN	0 % 0 CPM	1% 1114 CPM	1% 171 CPM
PE	0% 0 CPM	2.6% 14479 CPM	9.4% 4459 CPM
NEUTRAL FATS	100% 2887 CPM	97% 536281 CPM	90% 42687 CPM
	2887 CPM TOTAL	553545 CPM TOTAL	47374 CPM TOTAL

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ABSTRACT:

The coalescence between secretory granules facilitates the extrusion of a viscous material from the acinar cells into the ductal system. In the present report, the interaction of granular membranes during the coalescence was evaluated in the human mucous cells situated within the submucosal layer surrounding the maxillary sinus. In contrast, the serous granules of the other cells do not engage in any aspect of coalescence prior to their release into the lumen. The first ultrastructural indication of the coalescence is the transformation of phospholipid layers from both juxtaposed membranes into the pentalamellar profile. Similar to the reactions of the membranes investigated during the intralysosomal digestion, the middle phospholipid zone resulting from either apposition or interdigitation of two molecular layers is wider than the peripheral osmiophilic segments. Subsequent lysis of the modified membrane proceeds from only one side of the pentalamellar arrangement. This decomposition suggests that only certain secretory granules contain, in addition to acidic mucosubstances, the lipolytic enzymes capable of analysis of phospholipid layers. Although the nature of the triggering mechanism which activates these enzymes is not thoroughly understood, it appears that the displacement of the phospholipid chains proceeds first along the ipsilateral membrane. After the ipsilateral membrane is completely digested, the remaining membrane between two granules shows a typical unit structure. The further hydrolysis of such a membrane preceeds the fusion of granular matrices. In addition, the broken ends of both pairs of phospholipid layers reorganize to become continuous with their respective internal and external counterparts. The ultrastructural evidence presented herein indicates that the interaction of biological membranes during the coalescence of secretory granules is similar to processes suggested in pinocytosis, the formation of secondary lysosomes, the condensation of secretory material adjacent to the Golgi apparatus, and many others. (Supported by Grant from The Council for Tobacco Research - USA, Inc.)

--1) Vidic, B. (1975). Proceedings of the Tenth International Congress of Anatomists, Tokyo, Japan, August 24-30. "Interaction of membranous phospholipid layers during coalescence between some secretory granules."

From the Department of Anatomy, Georgetown University School of Medicine, Washington, D.C.

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